Differential expression of selenoproteins by human skin cells and protection by selenium from UVB-radiation-induced cell death

Teresa S. RAFFERTY¹*, Roderick C. MCKENZIE*, John A. A. HUNTER*, A. Forbes HOWIE†, John R. ARTHUR‡, Fergus NICOL‡ and Geoff J. BECKETT†

*Department of Dermatology, University of Edinburgh, Lauriston Place, Edinburgh EH3 9YW, Scotland, U.K., †Department of Clinical Biochemistry, University of Edinburgh, Lauriston Place, Edinburgh EH3 9YW, Scotland, U.K., and ‡Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, Scotland, U.K.

The generation of reactive oxygen species has been implicated as part of the mechanism responsible for UVB-radiation-induced skin damage. In mice, evidence suggests that increased dietary selenium intake may protect skin from many of the harmful effects of UVB radiation. We sought to determine the selenoprotein profile of cultured human skin cells and whether selenium supplementation could protect keratinocytes and melanocytes from the lethal effects of UVB radiation. Labelling experiments using [⁷⁵Se]selenite showed qualitative and quantitative differences in selenoprotein expression by human fibroblasts, keratinocytes and melanocytes. This was most noticeable for thioredoxin reductase (60 kDa) and phospholipid glutathione peroxidase (21 kDa); these proteins were identified by Western blotting. Despite these differences, we found that a 24 h preincubation

with sodium selenite or selenomethionine protected both cultured human keratinocytes and melanocytes from UVB-induced cell death. With primary keratinocytes, the greatest reduction in cell death was found with 10 nM sodium selenite (79 % cell death reduced to 21.7 %; $P < 0.01$) and with 50 nM selenomethionine (79 % cell death reduced to 13.2 %; $P < 0.01$). Protection could be obtained with concentrations as low as 1 nM with sodium selenite and 10 nM with selenomethionine. When selenium was added after UVB radiation, little protection could be achieved, with cell death only being reduced from 88.5 % to about 50 % with both compounds. In all of the experiments sodium selenite was more potent than selenomethionine at providing protection from UVB radiation.

INTRODUCTION

The prevalence of non-melanoma skin cancer is increasing, and cumulative ultraviolet B radiation (UVB) (280–320 nm) dose is generally accepted as being one of the most important aetiological factors. UVB is a complete carcinogen; it can both initiate and promote carcinogenesis. Two of its major effects are DNA damage and local and systemic immune suppression [1,2]. The generation of free radicals [3] and cytokines [4] has been implicated as being partly responsible for these effects. The skin contains many enzyme systems to prevent free-radical-induced damage, including catalase, superoxide dismutase and selenium (Se)-containing glutathione peroxidases (GPXs). Catalase and superoxide dismutase activity are decreased following UVB exposure, while GPX appears to be resistant to these effects [5].

There is much evidence to suggest that Se has an important role in protecting skin from the harmful effects of UVB. In mice, Se supplementation can substantially decrease the amount of skin damage, tumour formation and overall mortality following UVB exposure [6,7]. In humans, subnormal Se status is associated with up to a 4-fold increased risk of developing skin cancer [8], and topical Se application, as selenomethionine, has been shown to protect subjects from acute skin damage following UVB exposure [9].

All the actions of Se were once thought to be exerted through cytosolic GPX, but it is now recognized that Se exerts many of its effects through the expression of a number of selenoproteins, in which Se is covalently linked within the protein as selenocysteine [10]. At least 30 selenoproteins have been identified by SDS/PAGE of ⁷⁵Se-labelled tissue. However, only approximately

14 have been characterized, including cytosolic GPX, phospholipid hydroperoxide GPX (PHGPX), extracellular GPX, selenoprotein-P, SP56, protein disulphide isomerase, thioredoxin reductase (TR) and the iodothyronine deiodinases [11,12]. The effects of UVB on the expression of these selenoproteins, or indeed their precise roles in influencing the cells' response to UVB, are not known. The family of GPXs detoxifies a wide range of lipid peroxides [13], and these enzymes have been implicated in protecting cells from UVB-induced free-radical damage. TR is an FAD-containing enzyme found in all organisms which, in conjunction with its substrate thioredoxin, forms a redox system which has multiple functions, including detoxification reactions [14–16].

Labelling experiments with ⁷⁵Se have shown clear tissue differences in the pattern of selenoprotein expression [17], but studies have not been performed on human skin. Such studies may indicate which selenoproteins have a role in protecting skin from the harmful effects of UVB.

Both inorganic and organic forms of Se are commonly used as Se supplements in experiments, although dietary Se is found predominantly as the organic forms selenomethionine and selenocysteine [18]. In humans, whole-body turnover studies indicate that selenomethionine and selenocysteine are re-utilized more efficiently than inorganic forms such as sodium selenite [19]. This occurs because selenomethionine can be substituted non-specifically into proteins at methionine residues. However, inorganic forms of Se may provide a more bioactive form of the trace element than selenomethionine and, in cultured thyrocytes, sodium selenite is retained and utilized by an energy-dependent process [20]. Studies have not been performed to compare the

Abbreviations used: UVB, ultraviolet B radiation; GPX, glutathione peroxidase; PHGPX, phospholipid hydroperoxide glutathione peroxidase; TR, thioredoxin reductase; DMEM, Dulbecco's modified Eagle's medium; EBSS, Earle's balanced salt solution.

¹ To whom correspondence should be addressed (e-mail tr@srv1.med.ed.ac.uk).

potency of sodium selenite and selenomethionine in protecting cultured human skin cells from UVB.

Here, for the first time, we define the profiles of selenoproteins expressed by cultured human keratinocytes, melanocytes and fibroblasts. We also show that sodium selenite and selenomethionine supplementation protects human melanocytes and keratinocytes *in vitro* from UVB-induced cell death.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, glutamine, dispase, trypsin, sodium pyruvate, keratinocyte serum-free medium, Earle's balanced salt solution (EBSS) and the culture vessels were purchased from Gibco BRL, Paisley, Renfrewshire, Scotland, U.K. [^{75}Se]Selenite (33 MBq/mg) was purchased from the University of Missouri, Columbia, MO, U.S.A. All other chemicals and reagents and the melanocyte culture media were purchased from Sigma-Aldrich, Poole, Dorset, U.K. Antibodies to rat liver TR and rat PHGPX were raised in rabbits using proteins purified as described previously [14,21].

Tissue culture

Cultures of the spontaneously transformed keratinocyte line HaCaT [22] (a gift from Professor N. Fusenig, Differenzierung und Carcinogenese, Deutsches Krebsforschungszentrum, Heidelberg, Germany), were grown in DMEM supplemented with 5% (v/v) foetal calf serum, 100 units/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin in a humidified 5% CO_2 atmosphere at 37 °C. Primary fibroblasts were cultured from forearm biopsies [23], grown out from underneath coverslips and cultured until confluent. These were grown in DMEM as above, but with 10% foetal calf serum.

Primary human keratinocytes and melanocytes were cultured from neonatal foreskins as described previously [24]. Briefly, the foreskin samples were incubated overnight in 0.1% dispase/DMEM. The epidermis was removed and dispersed in trypsin (0.5 g/l) and EDTA (6.8 μM) for 15 min. Cells were then plated out on fibronectin-coated plates and grown in either keratinocyte or melanocyte medium. After two passages, the cultures were composed predominantly (> 95%) of either keratinocytes or melanocytes respectively. The cells were used at passages 3 or 4. The Se content of each culture medium was determined by acid digestion followed by fluorimetric analysis [25] and the following results found: melanocyte medium, 28 nM; keratinocyte medium, 8.9 nM. No Se was detected in the DMEM with 5 or 10% foetal calf serum added.

Determination of selenoprotein profiles

Profiles of selenoproteins expressed by the various primary skin cells were determined by radiolabelling with [^{75}Se]selenite as described previously [26]. Briefly, confluent cultures of cells in 75 cm^2 flasks were labelled with 0.02 MBq/ml [^{75}Se]selenite by incubation for 72 h. Previous studies have shown that ^{75}Se uptake and incorporation into selenoproteins by cells reaches a plateau at approx. 24 h, and therefore cells were labelled for 72 h to ensure that a steady state had been reached. Medium was decanted and cells were washed twice with EBSS before being harvested into EBSS by scraping, followed by centrifugation at 2000 *g* for 10 min. Cells were resuspended in 60 mM Tris buffer, pH 7.8, containing 1 mM dithiothreitol and 1 mM EDTA, and lysed by sonication. Equal amounts of protein (25 μg) were

loaded on to SDS/PAGE (12% gels) and proteins were separated by electrophoresis. The gels were dried and the labelled selenoproteins were quantified using a molecular PhosphorImager (model GS-525; Bio-Rad, Hemel Hempstead, Herts., U.K.) and visualized by autoradiography using Kodak X-OMAT XAR-5 film. Selenoprotein profiles were also determined in primary melanocytes, keratinocytes and fibroblasts which had either (1) been prelabelled with ^{75}Se for 24 h prior to being exposed to 200 J/m 2 UVB and then harvested 24 h post-exposure, or (2) been exposed to 200 J/m 2 UVB, then labelled with ^{75}Se and harvested 24 h later.

Western blot analysis was used to identify TR and PHGPX. The proteins were separated by SDS/PAGE and were transferred to Immobilon P membranes. The blot was probed with affinity-purified polyclonal antibodies to rat liver TR and developed using chemiluminescence or coupled alkaline phosphatase reagents [27]. The blots were also probed with purified antibodies to rat PHGPX.

Protein determination

The protein content of the sonicated samples was determined using the Bradford dye-binding method [28] adapted for use on a Cobas Fara centrifugal analyser (Roche Diagnostics, Welwyn Garden City, U.K.).

UV irradiation

To determine the lethal UVB dose for the various cell types, cells were grown to 70% confluence; the medium was then removed and set aside, and the monolayer was covered with 1 ml of PBS. Then the cells were irradiated with broadband UVB from a bank of two TL-20W/12 lamps (Philips, Croydon, U.K.) with an output range of 270–350 nm (peak at 308 nm). The irradiance was 80 mW/cm 2 at a distance of 30 cm [24]. To determine the UVB dose that killed 80% of the cells, doses of between 480 and 1200 J/m 2 were given. The original medium was replaced, and cell viability was determined by cell counts on a haemocytometer using Trypan Blue (0.4%, w/v) exclusion, 48 h after irradiation.

Effects of selenium supplementation on cell viability and growth

Cells grown in six-well dishes to 70% confluence were treated with sodium selenite or selenomethionine (1 nM–10 μM) and incubated for 72 h. The toxicity of these compounds was determined by assessing cell viability using Trypan Blue exclusion. Growth stimulation was assessed by counting cells using a Coulter counter (model DN; Coulter Electronics Ltd., Harpenden, U.K.).

Protective effect of selenium supplementation on cells treated with UVB

Cells cultured in six-well dishes until 70% confluent were treated with sodium selenite or selenomethionine (1 nM–1 μM), and incubated for 24 h. The medium was removed and set aside, and 1 ml of PBS was added to each well immediately prior to the cells being exposed to UVB. Typically the cells were exposed to UVB for 1.5–2 min (720–960 J/m 2 ; the dose given depended on the cell type used), which resulted in approx. 80% cell death 48 h post-irradiation, in cultures where no sodium selenite or selenomethionine had been added. After UVB exposure, PBS was removed and the original culture medium was replaced. Viability was determined by Trypan Blue exclusion 48 h post-UVB exposure.

In a second series of experiments, cells were treated with sodium selenite or selenomethionine immediately after UVB exposure (without the 24 h pretreatment with Se). The rest of the experiment was performed as above.

All experiments were carried out twice (with similar results), using triplicate samples for each data point. ANOVA and the *post hoc* test Bonferroni were used to analyse the data; values for $P < 0.05$ were deemed to be significant. To compare the differences between sodium selenite and selenomethionine, ANOVA and the *post hoc* test Scheffé were used; again values for $P < 0.05$ were deemed significant.

RESULTS

Selenoprotein profiles in cultured melanocytes, fibroblasts and keratinocytes

Primary keratinocytes, melanocytes and fibroblasts were found to have clearly different selenoprotein profiles (Figures 1 and 2). Each cell type showed up to 10 major selenoproteins (molecular mass ranging from approx. 10 kDa to 60 kDa), but many minor selenoproteins were also expressed. Of particular note was the variable expression between cell types of the 21 kDa and 60 kDa selenoproteins. Western blotting identified the 21 kDa and 60 kDa selenoproteins as being PHGPX and TR respectively (results not shown). The 60 kDa ^{75}Se -labelled bands in each cell line corresponded exactly in mobility and relative intensity to the bands revealed by Western blotting. The Western blot was not sensitive enough to detect PHGPX directly; however, a standard of pure PHGPX had exactly the same mobility as the 21 kDa ^{75}Se -labelled band. The greatest expression of TR and PHGPX was seen in fibroblasts, with keratinocytes expressing relatively little of these selenoproteins. PHGPX and TR expression was greater in melanocytes than keratinocytes, but not as great as in fibroblasts. At 24 h following exposure to UVB, no change in the expression of any of the selenoproteins could be observed (results not shown).

Figure 1 Selenoprotein expression by primary human skin cells

Lane 1, melanocytes; lane 2, keratinocytes; lane 3, fibroblasts. Cells were labelled with [^{75}Se]selenite (0.02 MBq) for 72 h, and the proteins were separated by SDS/PAGE and visualized by autoradiography.

Figure 2 Selenoprotein profile analysis of primary human skin cells

A PhosphorImager was used to quantify the radioactivity of the selenoproteins expressed by the primary skin cells. The protein gels were scanned overnight on an imager and the profiles of selenium-containing proteins were generated. The molecular masses are shown on the bottom profile.

Table 1 Effects of sodium selenite and selenomethionine on the viability of primary human keratinocytes

Primary human keratinocytes were incubated with sodium selenite or selenomethionine for 72 h, and the viable cells were counted using a haemocytometer and Trypan Blue exclusion. Control cells had no Se added to them. At least 100 cells were counted. Results are means $\pm$ S.E.M. ($n = 3$). Significant differences from control: * $P < 0.05$, ** $P < 0.01$.

Dose (μM)	Cell death (%)	
	Sodium selenite	Selenomethionine
Control	1.8 ± 1.8	1.8 ± 1.8
0.1	1.3 ± 1.8	1.8 ± 1.8
0.2	6.5 ± 1.2	1.2 ± 0.8
1	$24.5 \pm 1.5^*$	1.5 ± 0.3
10	$56.0 \pm 5.4^{**}$	5.4 ± 2.1

Effect of Se supplementation on cell growth and cell viability

In cultured primary keratinocytes, sodium selenite or selenomethionine had no apparent growth-stimulating activity (results not shown), and sodium selenite was only significantly toxic at concentrations of 1 μM or above (Table 1). Selenomethionine was not found to be toxic at concentrations up to 10 μM (Table 1).

Figure 3 Effect of sodium selenite or selenomethionine pretreatment on the viability of primary human keratinocytes after exposure to UVB

Primary keratinocytes were pretreated with sodium selenite or selenomethionine for 24 h; then the medium was replaced with PBS and the cells were exposed to a dose of UVB (960 J/m²). The original medium was returned and the cells were incubated for 48 h before cell counts were performed using a haemocytometer and Trypan Blue exclusion. Control cells received no UVB or Se supplement. At least 100 cells were counted. Results are given as percentage cell death (means $\pm$ S.E.M., $n = 3$). Significant difference from cells not treated with Se, but exposed to UVB: * $P < 0.05$; ** $P < 0.01$. Significant differences between the sodium selenite and selenomethionine results are indicated by † $P < 0.05$.

Similar results were found in primary melanocytes and HaCaT cells (not shown).

Effect of selenium in decreasing UVB-induced cell death

The dose of UVB used (960 J/m²) produced 80 % cell death in primary keratinocytes 48 h after exposure. The addition of sodium selenite or selenomethionine, prior to exposure to UVB, prevented a significant amount of cell death, but the protective effect was significantly different between the two compounds (Figure 3). A protective effect with sodium selenite was seen with doses from 1 to 200 nM, with an optimal protective dose of 10 nM. Selenomethionine was less potent than sodium selenite in protecting cells from UVB-induced damage, with a higher concentration of selenomethionine than sodium selenite being required to give significant protection from UVB-induced damage. No significant protective effect of selenomethionine was observed below 10 nM. Maximal protection using selenomethionine was found at 50 nM, with concentrations in the range 10 nM–1 μ M offering significant protection. As with sodium selenite, the protection afforded by selenomethionine started to decrease at 1 μ M.

Melanocytes were more sensitive to UVB than were keratinocytes, and a UVB dose of 720 J/m² was sufficient to kill 80 % of the cells. With melanocytes, the effects of Se were similar to those seen with keratinocytes, with maximal protection being achieved for sodium selenite and selenomethionine at concentrations of 10 nM and 100 nM respectively, with little or no protection found at 1 μ M (Table 2). In the keratinocyte cell line HaCaT, a dose of 960 J/m² UVB was required to kill 80 % of the cells, and maximal protection with Se was found at 10 nM sodium selenite and 100 nM selenomethionine (Table 2).

In experiments in which sodium selenite or selenomethionine

Table 2 Effects of selenomethionine and sodium selenite pretreatment on the viability of primary human melanocytes and the human keratinocyte cell line HaCaT after exposure to UVB

Primary melanocytes and HaCaT cells were pretreated with sodium selenite or selenomethionine for 24 h prior to the medium being replaced with PBS and the cells being exposed to a dose of UVB (melanocytes, 720 J/m²; HaCaT cells, 960 J/m²). The original medium was returned and the cells incubated for 48 h before cell counts were performed using a haemocytometer and Trypan Blue exclusion. Control cells received no UVB or Se supplement. At least 100 cells were counted. Results are the means $\pm$ S.E.M. ($n = 3$). Significant differences from cells not treated with Se, but exposed to UVB: * $P < 0.05$; ** $P < 0.01$. Significant differences between the sodium selenite and selenomethionine results are indicated by † $P < 0.05$.

Dose (nM)	Cell death (%)			
	Sodium selenite		Selenomethionine	
	Melanocytes	HaCaT cells	Melanocytes	HaCaT cells
Control	7.7 $\pm$ 1.6	4.3 $\pm$ 4.3	7.7 $\pm$ 1.6	4.3 $\pm$ 4.3
0	82.6 $\pm$ 1.4	86.8 $\pm$ 1.1	82.6 $\pm$ 1.4	86.8 $\pm$ 1.1
1	57.7 $\pm$ 6.4*†	57.6 $\pm$ 2.9*†	79.3 $\pm$ 1.7	79.4 $\pm$ 3.8
10	10.3 $\pm$ 0.9***†	19.5 $\pm$ 1.4***†	72.4 $\pm$ 2.3*	64.3 $\pm$ 2.2*
50	22.5 $\pm$ 1.5***†	35.4 $\pm$ 1.9**	26.9 $\pm$ 1.5**	23.8 $\pm$ 4.5**
100	29.2 $\pm$ 1.1**	45.2 $\pm$ 2.8*	14.3 $\pm$ 4.9**	11.6 $\pm$ 3.9***†
200	34.4 $\pm$ 3.5**	60.4 $\pm$ 2.2*	30.3 $\pm$ 3.4**	39.5 $\pm$ 3.1***†
1000	75.4 $\pm$ 3.4	86.7 $\pm$ 2.3	65.6 $\pm$ 3.3*	75.2 $\pm$ 3.4

Figure 4 Effects of sodium selenite or selenomethionine added after the exposure of primary human keratinocytes to UVB

The medium on primary keratinocytes was replaced with PBS prior to the cells being exposed to a dose of UVB (960 J/m²). Then the original medium was returned, with either sodium selenite or selenomethionine added, and the cells were incubated for 48 h before cell counts were performed using a haemocytometer and Trypan Blue exclusion. Control cells received no UVB or Se supplement. Results are given as percentage cell death (means $\pm$ S.E.M., $n = 3$). Significant differences from cells not treated with Se, but exposed to UVB: * $P < 0.05$, ** $P < 0.01$.

was added to primary keratinocytes immediately after UVB exposure, protection was still achieved, but the protection was modest and required higher doses of Se compared with protection achieved by adding sodium selenite or selenomethionine to cells 24 h prior to UVB exposure (Figure 4). Maximal protection was achieved at 50 nM sodium selenite and 200 nM selenomethionine. The level of protection was much less (2–3-fold decrease in cell death) for the cells treated with Se post-UVB compared with that

for the cells pretreated with Se for 24 h (5–6-fold decrease in cell death) (Figures 3 and 4).

DISCUSSION

The results presented here show that preincubation of primary cultures of keratinocytes, melanocytes or the HaCaT cell line with Se for 24 h can provide all of these cell types with significant protection from UVB-induced cell death (Figure 3; Table 2). The chemical form of Se added to cells was an important factor in determining the minimal and optimal doses of Se required to provide protection from UVB. Selenite was more potent than selenomethionine at conferring protection, illustrated by the finding that, in melanocytes, keratinocytes and HaCaT cells, significant protection was achieved with a dose of 1 nM selenite, whereas no significant protection was achieved with selenomethionine below concentrations of 10 nM. Concentrations of Se that conferred maximal protection to cells also varied with the chemical form of Se. With selenite, maximal protection was achieved at a concentration of 10 nM in all cell types, whereas, with selenomethionine, maximal protection was achieved at concentrations of 50 nM, 100 nM and 100 nM for keratinocytes, HaCaT cells and melanocytes respectively (Figure 3; Table 2). The observation that a similar degree of protection conferred by Se addition was found with the HaCaT keratinocyte cell line and primary keratinocytes suggests that the phenomenon is a general one among skin cell types.

We did not investigate the effects of Se on fibroblasts, as malignancies in the skin arise from keratinocytes and melanocytes only. However, Richard et al. [29] have shown that fibroblasts can be protected from broadband UVB by selenium.

It is widely held that Se exerts many of its biochemical actions through the expression of specific selenoproteins in which Se is inserted as specific selenocysteine residues encoded by a TGA triplet. Insertion of Se at these TGA-directed sites requires Se to be presented in a chemically active form similar to selenide, and current evidence indicates that selenite is a more potent precursor of selenide than is selenomethionine [30]. Furthermore, although whole-body turnover studies in humans have shown that selenomethionine is re-utilized by cells more efficiently than inorganic forms, this appears to arise because selenomethionine can be substituted into proteins non-specifically for methionine. When replacing methionine residues, selenomethionine appears to have no bioactivity [31]. Further evidence for the increased bioactivity of selenite over selenomethionine comes from the observation that selenite, but not selenomethionine, can be converted within the cell into selenodiglutathione, a form of Se which appears able to regulate apoptosis [32].

Although selenite was more potent than selenomethionine at conferring protection from UVB damage, our data clearly showed that selenite was much more toxic to cells than was selenomethionine. Thus 1 μ M selenite killed approx. 25 % of cells, and at a dose of 10 μ M more than 50 % of the cells were destroyed; this contrasts with selenomethionine, which showed no toxicity at any of the doses used in the study (Table 1). While selenite may itself be directly toxic to cells, a study has shown that the metabolite selenodiglutathione is also a potent cytotoxic agent [32]. Although both Se compounds conferred dose-dependent protection from UVB damage up to approx. 100 nM, protection appeared to be diminished in all cell types when the dose of Se was further increased to 1 μ M. This loss of protection with increasing concentration of selenite is most likely to be due to a direct toxic effect of selenite and its metabolite selenodiglutathione when present at high concentrations. For selenomethionine, the mechanism behind the loss of protection at

higher doses is unknown, since selenomethionine was not toxic at any of the concentrations used (Table 1). It is possible that UVB exposure might modify selenomethionine to produce a toxic metabolite, but we could find no evidence of increased toxicity in a solution of selenomethionine irradiated with UVB (results not shown).

We have shown that preincubation with Se can prevent UVB-induced damage; the mechanism by which this occurs is not clear. Se did not exert a protective effect through altering cell growth patterns at the doses that were protective. If Se was added immediately after UVB exposure, some protection was achieved, but this effect was small compared with the protection achieved if the cells were preincubated with the trace element (Figure 4). These data suggest that Se may be acting through incorporation into selenoproteins rather than by a direct antioxidant chemical action.

The family of GPXs are clearly potential candidates for selenoproteins that may mediate the protective effects of Se, since these enzymes are capable of detoxifying hydrogen peroxide, lipid hydroperoxides and phospholipid hydroperoxides which are produced during UVB exposure. TR is a selenoenzyme which has multiple functions and can act through its ability to reduce oxidized thioredoxin [14] or its ability to directly reduce hydrogen peroxide and lipid hydroperoxides in the presence of NADPH [33]. Thus TR may play an important role in protecting cells from peroxidative damage. However, labelling of tissues with [^{75}Se]selenite has revealed more than 30 selenoproteins, and the pattern of selenoprotein expression is quite different between tissues. It is thought that some of these selenoproteins may also be involved in detoxification reactions and in modifying cell growth or cell death [30].

The expression of the 21 and 60 kDa selenoproteins showed the most diversity between cell types. These selenoproteins were identified by Western blotting to be PHGPX and TR. We found that keratinocytes expressed much lower levels of TR and PHGPX than melanocytes, and yet keratinocytes were found to be more resistant to UVB-induced cell death than melanocytes; typically, a UVB dose of 720 J/m² was required to produce 80 % cell death in melanocytes, while a dose of 960 J/m² was required to produce 80 % cell death in keratinocytes. Furthermore, despite these differences in selenoprotein expression between the two cell types, keratinocytes and melanocytes showed a similar dose requirement for selenium to obtain maximal protection. While it could be argued that these observations imply that the selenoproteins TR and PHGPX are not involved in protection, this is not necessarily the case. The susceptibility of a cell to UVB-induced damage will depend on the total expression of a wide range of antioxidant enzymes and DNA repair mechanisms, and not necessarily on differences in the expression of a single specific selenoprotein. Thus conclusions regarding the protective role of a specific selenoprotein made on the basis of comparison of the susceptibility of different cell types to UVB-induced cell death may be misleading; experiments that relate to the susceptibility of a cell to UVB-damage after modifying the expression of a single selenoprotein within a single cell type would provide more conclusive data.

We have shown that Se can protect both keratinocytes and melanocytes from UVB-induced damage, and such protection can be achieved using very low concentrations of Se, particularly when presented as sodium selenite. Intake of Se in the U.K. has fallen considerably over the past two decades; at present, the average intake is only about 40 % of the recommended daily allowance (75 μ g/day) [34]. It is possible that a contributing factor to the increase in the incidence of skin cancer of all types seen in the U.K. [35] may, in part, be due to this decline in Se

status. Our data from isolated cells would suggest that, as in the mouse [6], increasing the Se intake of humans could afford protection from the harmful effects of UVB radiation. Furthermore, our results suggest that, if supplements of Se were to be given, inorganic forms such as selenite may be more potent at providing this protection than organic forms such as selenomethionine.

We thank the staff at the Royal Sick Children's Hospital (Edinburgh, Scotland, U.K.) for collecting skin tissue, Ms. M. McCann at the Scottish Blood Transfusion Laboratories (Edinburgh, Scotland, U.K.) for kindly providing purified fibronectin, and Professor N. Fusenig (Differenzierung und Carcinogenese, Deutsches Krebsforschungszentrum) for kindly providing the HaCaT cell line. T.S.R. was in receipt of an Edinburgh University Medical Faculty scholarship. The work was funded by grants from the Sir Stanley and Lady Davidson Trust, the British Foundation for Skin Research and the Foundation For Skin Research. Support for J.R.A. and F.N. was by the Scottish Office Agriculture and Environment and Fisheries Department (SOAFED).

REFERENCES

- Ananthwaswamy, H. and Piercall, W. (1990) *Photochem. Photobiol.* **52**, 1119–1136
- Kripke, M. (1990) *Photochem. Photobiol.* **52**, 919–924
- Danno, K., Horio, T., Takigawa, M. and Imamura, S. (1984) *J. Invest. Dermatol.* **83**, 166–168
- Ullrich, S. (1995) *Photochem. Photobiol.* **62**, 389–401
- Shindo, Y., Witt, E., Han, D., Tzeng, B., Aziz, T., Nguyen, L. and Packer, L. (1994) *Photodermatol. Photoimmunol. Photomed.* **10**, 183–191
- Pence, B., Delver, E. and Dunn, D. (1994) *J. Invest. Dermatol.* **102**, 759–761
- Stewart, M., Cameron, G. and Pence, B. (1996) *J. Invest. Dermatol.* **106**, 1086–1089
- Clark, L., Graham, G., Crounse, R., Grimson, R., Hulka, B. and Shy, C. (1989) *Nutr. Cancer.* **6**, 13–21
- Burke, K., Burford, R., Combs, G., French, I. and Skeffington, D. (1992) *Photodermatol. Photoimmunol. Photomed.* **9**, 52–57
- Cone, J., Martin Del Rio, R., Davis, J. and Stadtman, T. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2659–2663
- Arthur, J., Bermanno, J., Mitchell, J. and Hesketh, J. (1996) *Biochem. Soc. Trans.* **24**, 384–387
- Burk, R. and Hill, K. (1993) *Annu. Rev. Nutr.* **13**, 65–81
- Rotruck, J., Pope, A., Ganther, H., Swanson, A., Hafeman, D. and Hoekstra, W. (1973) *Science* **179**, 588–590
- Holmgren, A. and Bjornstedt, M. (1995) *Methods Enzymol.* **252**, 199–208
- Tamara, T. and Stadtman, T. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1006–1011
- Gladyshev, V., Jeang, K. and Stadtman, T. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 6146–6151
- Behne, D., Hilmert, H., Scheid, S., Gessner, H. and Elgar, W. (1988) *Biochim. Biophys. Acta* **966**, 12–21
- Daniels, L. (1996) *Biol. Trace Elem. Res.* **54**, 185–199
- Swanson, C., Patterson, B., Levander, O., Veillon, C., Taylor, P. and Helzlsouer, K. (1991) *Am. J. Clin. Nutr.* **54**, 917–926
- Beech, S., Walker, S., Arthur, J., Nicol, F. and Beckett, G. (1993) in *Trace Elements in Man and Animals* (Anke, M., Meissner, D. and Mills, F., eds.), pp. 1062–1066, Verlag Media Touristik, Germany
- Roveri, A., Maiorino, M., Nisii, C. and Ursini, F. (1994) *Biochim. Biophys. Acta* **1208**, 211–221
- Boukamp, P., Petrussevska, R., Breitkreutz, D., Hornung, J., Markham, A. and Fusenig, N. (1988) *J. Cell Biol.* **106**, 761–771
- Sly, W. and Grubb, J. (1979) *Cell Culture, Methods in Enzymology* (Jakoby, W. and Paston, I. eds.), pp. 440–450, Academic Press, London
- Kondo, S., Kono, T., Sauder, D. and McKenzie, R. (1993) *J. Invest. Dermatol.* **101**, 690–694
- Boyne, R. and Arthur, J. (1986) *J. Nutr.* **116**, 816–822
- Howie, A. F., Walker, S., Akesson, B., Arthur, J. and Beckett, G. (1995) *Biochem. J.* **308**, 713–717
- McLeod, R., Ellis, E., Arthur, J., Neal, G., Judah, D., Manson, M. and Hayes, J. (1997) *Cancer Res.* **57**, 4257–4266
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Richard, M., Guiraud, P., Cadi, R., Monjo, A., Amblard, P. and Favier, A. (1990) *Nouv. Dermatol.* **9**, 1–7
- Sunde, R. (1990) *Annu. Rev. Nutr.* **10**, 451–474
- Waschulewski, I. and Sunde, R. (1998) *J. Nutr.* **118**, 367–374
- Wu, L., Lanfear, J. and Harrison, P. (1995) *Carcinogenesis* **16**, 1579–1584
- Bjornstedt, M., Hamberg, M., Kumer, S., Xue, J. and Holmgren, A. (1995) *J. Biol. Chem.* **270**, 11761–11764
- Rayman, M. (1997) *Br. Med. J.* **314**, 387–388
- Ko, C., Walton, S., Keczkes, K., Bury, H. and Nicholson, C. (1994) *Br. J. Dermatol.* **130**, 269–272